DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date . APR 28 1999

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From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	*Haematoccoccus pluvialis* (*Haematoccoccus* algae)
Firm:	Cyanotech Corporation
Date Received by FDA:	March 22, 1999
90-day Date:	June 19, 1999

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after June 19, 1999.

Robert J. Moore

Robert J. Moore, Ph.D.

95S-0316

RPT 45

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

APR 28 1999

176 99 MY -5 A9:37

R. Todd Lorenz, Ph.D.
Scientific Director
Cyanotech Corporation
73-4460 Queen Kaahumanu Highway
#102
Kailua-Kona, Hawaii 96740

Dear Dr. Lorenz:

This is in response to your letter to the Food and Drug Administration (FDA) dated March 18, 1999, making a submission for a new dietary ingredient pursuant to 21 U.S.C. 350b(a)(2) (section 413 of the Federal Food, Drug, and Cosmetic Act (the Act)) and 21 CFR 190.6. Your letter notified FDA of your intent to market products containing the ingredient *Haematococcus pluvialis* (*Haematococcus* algae).

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement that contains a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 342(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

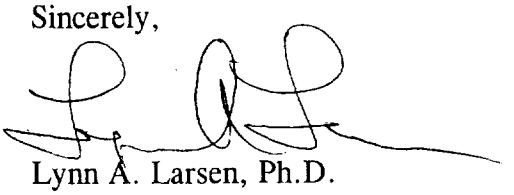
Your submission contained information that you believe establishes that the new dietary ingredient *Haematococcus* algae, when used under the conditions recommended or suggested in the labeling of the dietary supplements, will reasonably be expected to be safe. The information in your submission does not meet the requirements of 21 CFR 190.6 because it does not include reprints or photostatic copies of references to published information offered in support of the notification (see 21 CFR 190.6(b)(4)). Moreover, FDA is unable to determine whether the scientific studies you cite provide an adequate basis for a conclusion that the dietary supplement will reasonably be

expected to be safe because the summaries you have provided are incomplete and do not include adequate information about the methods used or the actual results of the studies. Finally, your submission states that the product contains canthaxanthin, a substance purported to be associated with adverse events in humans when ingested (see Bluhm et al., *JAMA* 1990; 264:1141-2; Boudreault et al., *Can. J. Opthalmol* 1983; 18:325-8; Ros et al., *Photodermatology* 1985; 2:183-5). However, your submission does not address this issue or the potential for ingested astaxanthin, which is similar to canthaxanthin, to result in such effects. You may submit an amended notification that cures the defects described above.

If you market your product without submitting an amended notification that meets the requirements of 21 CFR 190.6, or less than 75 days after submitting such a notification, your product is considered adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Please contact us if you have any questions concerning this matter.

Sincerely,



Lynn A. Larsen, Ph.D.
Director
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

CYANOTECH CORPORATION

6-4279

ISO 9002-94 CERTIFIED
QUALITY MANAGEMENT SYSTEM

RECEIVED
3/22/99

Thursday, March 18, 1999

Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street SW
Washington DC 20204

RE: New Dietary Ingredient Notification for *Haematococcus* algae

Dear Administrator,

As prescribed by 21 CFR Subpart B 190.6, please be advised of this New Dietary Ingredient Notification for *Haematococcus* algae. We plan to market *Haematococcus* algae as a human supplement 75 days after the acknowledgement of receipt of this notice, unless otherwise instructed by your agency. Please find enclosed one original and two copies of this notification. There are three copies of each attachment as well.

We presently produce a similar *Haematococcus* algae product for Japan and other countries for salmonids and other aquatic species (NatuRose™ *Haematococcus* algae meal). This *Haematococcus* algae meal product is in the final stages of review as a color additive in salmonid fish feeds by the Center for Food Safety and Applied Nutrition office of the FDA (CAP 8CO256). Much of the safety summary has been taken from this Color Additives petition, Part D, 21 CFR 71.1 (c). The committee has already scrutinized the safety section for our petition without concerns. I must note, however, that the following proposed human supplement form of *Haematococcus* algae will not contain ethoxyquin, as does the formula for fish feeds.

With respect to the other specifics of 21 CFR Ch.1 Subpart B 190.6:

Part (b)(1): The name and address of the manufacturer of the proposed new dietary supplement, *Haematococcus* algae is:

73-4460 Queen Kaahumanu Highway, #102 • Kailua-Kona, Hawaii 96740 • Phone (808) 326-1353 • Fax: (808) 329-3597

CYANOTECH CORPORATION

Cyanotech Corporation
73-4460 Queen Kaahumanu Highway Suite 102
Kailua-Kona, HI USA
96740
Ph: 808-326-1353
FAX: 808-329-3597

Part (b)(2): The name of the dietary ingredient subject to pre-market notification including Latin binomial name:

Haematococcus pluvialis otherwise known as *Haematococcus* algae

Part (b)(3): A description of the dietary supplement including the level of the new dietary ingredient in the dietary supplement and the conditions of use suggested in the labeling of the dietary supplement.

Haematococcus algae is a spray-dried powder of *Haematococcus pluvialis.* A full description of *Haematococcus* algae is attached and entitled "A Technical Review of *Haematococcus* Algae".

The *Haematococcus* algae powder may be manufactured with 1% of food grade rosemary oil. Typically, a 1:1 mixture will be made with *Haematococcus* algae meal and a food grade vegetable oil and then manufactured into soft gels or capsules. The *Haematococcus* algae powder may also be tableted or manufactured into soft gels or capsules without food grade vegetable oil. Typically, 300 mg of *Haematococcus* algae powder will be packaged into each capsule, soft gel or tablet. *Haematococcus* algae powder may also be blended with food grade *Spirulina* or other non-adulterated dietary supplements and either tableted or packaged into soft gels or capsules. The manufactured soft gels, capsules or tablets will typically be packaged into bottles or blister packs.

The labeling of the package will reflect the amount of *Haematococcus* algae contained in each gel cap or capsule, the type of oil(s) if contained, and other components such as *Spirulina* that may be blended in. Other appropriate labeling information will be printed according to the current guidelines. The conditions of use on the label will suggest 2 capsules per day, equivalent to 600 mg of *Haematococcus* algae powder.

Part (b)(4): The history of use or other evidence of safety establishing that the dietary ingredient will reasonably be expected to be safe under the conditions suggested on the label.

CYANOTECH CORPORATION

We have enclosed detailed historical literature search and background information on *Haematococcus* algae in the attached document entitled "A Technical Review of *Haematococcus* Algae". Additionally, we have prepared a summary of the safety studies that have been conducted with *Haematococcus* algae in the attached document entitled "*Haematococcus* Algae Safety Summary". *Haematococcus* algae has been approved in Japan for use in foods and fish feeds.

We contend that this product should not be considered or deemed adulterated as we meet the evidence of safety under the Federal Food, Drug, and Cosmetic Act Sec 413 (350b) (a) (2). We claim that under the conditions recommended in the labeling of the dietary supplement, *Haematococcus* algae can reasonably expected to be safe. The product will be manufactured under current food GMP conditions with quality assurance controls during and after processing.

Thank you for your attention to this matter. If you have any questions please do not hesitate to contact me at 808-326-1353 (ext. 115).

Kind regards,

R. Todd Lorenz, Ph.D.
Scientific Director

CC: Dr. Gerry Cysewski, CEO Cyanotech Corporation

73-4460 Queen Kaahumanu Highway, #102 • Kailua-Kona, Hawaii 96740 • Phone (808) 326-1353 • Fax: (808) 329-3597

A Technical Review of *Haematococcus* Algae

History, Distribution and Classification of *Haematococcus pluvialis*

Observations of *Haematococcus* began in 1797 by Girod-Chantrans and were continued by other Europeans. The first description of *Haematococcus pluvialis* was conducted by Flotow in 1844, and in 1851 Braun added to the details and corrected a few errors of earlier observations. Herrick published some brief comments in 1899 on the life history of *Haematococcus*, noting the alternation of lifecycle between resting cells and motile cells.

The first extensive description of the life history of *Haematococcus* in English was by T.E. Hazen in 1899 in a published report of the Torrey Botanical Club. He noted that the algae is usually found as a blood-red crust adhering to the sides of urns or shallow pools near the ocean which were periodically filled with water. He went on to describe the life history of the alga through a red resting stage and green swimming stage followed again by a red resting stage. At this time the chemical nature of the red coloring matter within the alga was unknown, but was given the name "haematochrom", and is now known as astaxanthin. Hazen reported that the alga "is reported as very common and widely distributed in Europe, where it is found from Scandinavia to Venice...the alga is distributed from Vermont to Texas and from Massachusetts to Nebraska and probably farther West."

A few years later, Peebles (1901a, 1909b) published a life history of the alga with detailed drawings of changes occurring in the "haematochrom" throughout the life cycle. In 1934, Elliot added details of the cellular morphology to the life history of the alga. During the life cycle four types of cells were distinguished: microzooids, large flagellated macrozooids, non-motile palmella forms; and haematocysts, which are large red cells with a heavy resistant cell wall. The macrozooids predominated in liquid cultures with sufficient nutrients, but when environmental conditions become unfavorable the palmella stage results, followed by the resistant haematocysts and the accumulation of astaxanthin. Subsequently, after being exposed to a nutrient-favorable environment, haematocysts give rise to motile microzooids that grow into palmella or macrozooid stages.

Pocock (1937 and 1961) described the distribution and life history of *Haematococcus* strains isolated in Africa. Almgren (1966) described the ecology and distribution of *Haematococcus* in Sweden, where the alga is found in ephemeral rain pools made of rock, generally of small dimensions and based upon firm material, impermeable to water. Droop (1961) also noted that that *Haematococcus* typically inhabited rock pools, often, though not necessarily, within a few feet of the sea.

The widespread occurrence of *Haematococcus* in temporary rather than permanent bodies of water is due, at lease in part, to the fact that such pools are usually free of other competing algae, and not to any inherent characteristic of the pools. *Haematococcus* is considerably better suited for survival under conditions of expeditious and extreme fluctuations in light, temperature and salt concentration than most algae, due to its rapid ability to encyst (Proctor, 1957a).

Haematococcus pluvialis, also referred to as *Haematococcus lacustris* or *Sphaerella lacustris*, is a ubiquitous green alga of the order Volvocales, family Haematococcaceae (Table 1). It is now known that the alga occurs in nature worldwide, where environmental conditions for its growth are favorable. No toxicity associated with *Haematococcus* has ever been reported in the literature.

Table 1: Classification

Haematococcus is an ubiquitous green algae classified as:

Phylum:	Chlorophyta
Class:	Chlorophyceae
Order:	Volvocales
Family:	Haematococcaceae
Genus:	Haematococcus
Species:	pluvialis

General Properties and Composition of *Haematococcus* algae

The general composition of *Haematococcus* algae consists of common carotenoids, fatty acids, proteins, carbohydrates, and minerals, and is listed in Table 2. Some physical characteristics are listed in Table 3.

Table 2: Typical Common Components of *Haematococcus* algae

	Minimum	Maximum	Mean
protein	17.30	27.16	23.62
carbohydrates	36.9	40.0	38.0
fat	7.14	21.22	13.80
iron (%)	0.14	1.0	0.73
moisture	3.0	9.00	6.0
magnesium (%)	0.85	1.4	1.14
calcium (%)	0.93	3.3	1.58
biotin (mg/lb)	0.108	0.665	0.337
L-carnitine (ug/g)	7.0	12	7.5
folic acid (mg/100g)	0.936	1.48	1.30
niacin (mg/lb)	20.2	35.2	29.8
pantothenic acid (mg/lb)	2.80	10.57	6.14
vitamin B1 (mg/lb)	<0.050	4.81	2.17
vitamin B2 (mg/lb)	5.17	9.36	7.67
vitamin B6 (mg/lb)	0.659	4.5	1.63

vitamin B12 (mg/lb)	0.381	0.912	0.549
vitamin C (mg/lb)	6.42	82.7	38.86
vitamin E (IU/lb)	58.4	333	186.1
ash	11.07	24.47	17.71

Table 3: Physical Characteristics *Haematococcus* Algae:

Color	Red to Dark red
Particle size	5-25 microns
Moisture	4-9%
Bulk density	
loose value	0.303-0.345 g/ml
tapped value	0.370-0.435 g/ml
astaxanthin	1.0%

The amino acid profile of *Haematococcus* algae is listed in Table 4.

Table 4: Typical Amino Acid Analysis of *Haematococcus* algae

	Minimum value	Maximum value	Mean
tryptophan	0.05	0.56	0.31
aspartic acid	1.37	2.31	1.89
threonine	0.78	1.24	1.04
serine	0.73	1.06	0.94
glutamic acid	1.70	2.39	2.19
proline	0.69	1.00	0.89
glycine	0.84	1.32	1.17
alanine	1.30	1.92	1.73
cysteine	0.16	0.21	0.19
valine	0.83	1.94	1.36
methionine	0.32	0.43	0.40
isoleucine	0.55	0.97	0.79
leucine	1.21	1.84	1.67
tyrosine	0.40	0.63	0.52
phenylalanine	0.61	1.05	0.90
histidine	0.48	0.76	0.61
lysine	0.75	1.32	1.13
arginine	0.81	1.34	1.07

Table 5 lists the individual fatty acids that are found in *Haematococcus* algae.

Table 5: Typical Fatty Acid Analysis of Haematococcus algae

Fatty Acid	Mean	Minimum	Maximum
C12:0 lauric	< 0.01	<0.005	0.01
C14:0 myristic	0.07	0.04	0.10
C16:0 palmitic	3.82	2.078	6.15
C16:1 palmitoleic	0.08	0.02	0.17
C17:0 margaric	0.03	0.01	0.03
C17:1 margaroleic	0.17	0.09	0.23
C18:0 stearic	0.27	0.14	0.46
C18:1 oleic	3.41	1.66	5.31
C18:2 linoleic	2.74	1.44	4.40
C18:3 linolenic	1.47	0.86	2.11
C18:3 gamma linolenic omega 6	0.21	0.09	0.29
C18:4 octadecatetraenoic	0.19	0.09	0.25
C20:0 arachidic	0.08	0.04	0.12
C20:1 gadoleic	0.04	0.01	0.08
C20:2 eicosadienoic	0.16	0.06	0.21
C20:3 eicosatrienoic gamma	0.06	0.02	0.09
C20:4 arachidonic	0.18	0.082	0.31
C20:5 eicosapentaenoic omega 3	0.08	0.031	0.18
C22:0 behenic	0.05	0.02	0.08
C24:0 lignoceric	0.03	0.013	0.05

Carotenogenesis and Astaxanthin of *Haematococcus pluvialis*

The pigment in *Haematococcus* was termed "haematochrom" until 1944 when Tisher identified the principal carotenoid as astaxanthin. Goodwin and Jamikorn (1954) identified the other pigments produced in *Haematococcus* during carotenogenesis. In 1954, Droop described the conditions governing astaxanthin formation and loss in *Haematococcus*. He showed that the action of light and carbon dioxide were dependent on one another, but that of organic carbon (such as acetate) is independent of light. Thus, astaxanthin formation could occur in the dark when energy is derived from organic carbon. Droop (1955a; 1955b) determined that the conditions for encystment and carotenogenesis in the alga were the same, and that the two phenomena usually occur together. Encystment and astaxanthin production can be induced by low nitrate or phosphate, high temperature or light, or the addition of sodium chloride in the culture medium (Boussiba and Vonshak, 1991, Kobayashi et al. , 1992, Fan et al. , 1994, Kakizono et al., 1992).

Sestak and Baslerova (1963) used paper chromatography to follow the changes in pigment composition of *Haematococcus* during encystment and carotenogenesis. They found

that astaxanthin precursors and chlorophyll decreased as astaxanthin accumulated. In 1976 Donkin used radioactively labeled acetate to determine that biosynthesis of astaxanthin occurs in *Haematococcus* through the intermediates beta-carotene, echinenone and canthaxanthin. The process of accumulation of astaxanthin in *Haematococcus* has been analyzed by optical and electron microscopes (Lang, 1968; Santos and Mesquita, 1984). In motile cells, astaxanthin first appears in small spherical inclusions (with no true limiting biomembrane) in the perinuclear cytoplasm, the pigment granules are not within any specific organelle or vesicle. In maturing cysts the pigment deposits increase in number and take on a variety of shapes. Coalescence of the globular granule result from increasing quantities of astaxanthin formed as the cell ages. In mature cysts the cytoplasm is almost uniformly red with no pigment in the nucleus or chloroplast.

Astaxanthin disperses towards the periphery of *Haematococcus* cells under light induction, and moves back towards the center after illumination is discontinued (Yong and Lee, 1991). No major quantitative or qualitative changes occur during this migration. Red cysts are more resistant to photoinhibition than green cysts, strongly indicating a photoprotective role for astaxanthin. The specific rate of astaxanthin accumulation is a function of the photon flux density *Haematococcus* cultures are exposed (Lee and Soh, 1991). Continuous illumination is most favorable for astaxanthin formation, and carotenoid content is correlated proportionally to light quantity. Other studies support the major role of astaxanthin accumulation in *Haematococcus* as being a form of protection against high light and oxygen radicals (Kobayashi et al., 1992a).

In nature, algae synthesize the carotenoid pigment astaxanthin and concentrate it in the food chain through zooplankton and crustaceans, which are prey for salmon, trout and other aquatic animals. The composition of astaxanthin esters in *Haematococcus* is similar to that of crustaceans, the natural dietary source of salmonids (Lambertsen, C. and O.R. Braekkan, 1971, Foss et al., 1987, Maoka, T. et al., 1985).

The astaxanthin molecule has two asymmetric carbons located at the 3 and 3" positions of the benzenoid rings on either end of the molecule. Different enantiomers of the molecule result from the exact way that the hydroxyl groups (-OH) are attached to the carbon atoms at these centers of asymmetry (Figure 1). If the hydroxyl group is attached so that it projects above the plane of the molecule it is said to be in the R configuration and when the hydroxyl group is attached to project below the plane of the molecule it is said to be in the S configuration. Thus the three possible enantiomers are designated R,R', S,S' and R,S' (meso).Free astaxanthin and its mono- and diesters from *Haematococcus* have optically pure (3S,3'S)-chirality (Grung et al., 1992 and Renstrom et al., 1981).

Astaxanthin, is biosynthesized through the isoprenoid pathway which is also responsible for the vast array of lipid soluble molecules such as sterols, steroids, prostaglandins, hormones, vitamins D, K and E. The pathway initiates at acetyl-Co-A and proceeds through phytoene, lycopene, β-carotene, and canthaxanthin before the last oxidative steps to astaxanthin. The astaxanthin biosynthetic pathway of *Haematococcus* is described in Figure 2. Fatty acids are esterified onto the 3' hydroxyl group(s) of astaxanthin after biosynthesis of the carotenoid, and allows it to have more solubility and stability in the cellular environment.

The carotenoid fraction of green vegetative cells consists of mostly lutein (75-80%) and β-carotene (10-20%). Whereas in red cysts, the predominate carotenoid is astaxanthin (Renstrom et al., 1981).

Astaxanthin is presently exempt from certification under the US 21 CFR part 73.35 as a color additive in fish feed, and *Haematococcus* algae meal is currently in the approval process by the Food and Drug Administration as a color additive for aquaculture feeds. *Haematococcus* algae meal has been approved in Japan as a natural food color and as a pigment for fish feeds. The formal descriptions of astaxanthin are presented in Table 6.

Table 6: Formal Descriptions of Astaxanthin

Chemical name:	3, 3'-dihydroxy-β,β,-carotene-4, 4' dione.
Molecular formula:	$C_{40}H_{52}O_4$
Molecular weight:	596.82
CAS number:	472-61-7
EINECS number	207-451-4

Quality Control Standards of *Haematococcus* Algae

GMP (Good Manufacturing Practice) is employed for the manufacture of *Haematococcus* algae. Pure cultures of the algae are cultivated employing Cyanotech's proprietary closed culture technology known as PhytoMax PCS (Pure Culture System) which automatically regulates pH and temperature, before transfer to open ponds for the final stage of the process. Under the proper stress conditions, *Haematococcus* encysts and produces high concentrations of carotenoids, which facilitates its own protection against light and oxygen. The carotenoid fraction of *Haematococcus* algae contains about 70% monoesters of astaxanthin, 10% diesters of astaxanthin, 5% free astaxanthin, and the remaining 15% consists of a mixture of β-carotene, canthaxanthin, lutein and other carotenoids (Figure 3). The production process includes a technique which "cracks" greater than 95% of the cells to enable maximum bioavailability. Because the process is biological, astaxanthin titer of individual batches may vary, thus total astaxanthin content is standardized to either 1.0% concentration (10,000 ppm) by blending of various lots in large stainless steel tumbler cones.

All media ingredients for the cultivation of the algae are food grade or higher quality. Reliable manufacturers that include specifications for heavy metals and other possible contaminants supply all nutrients. No solvents, pesticides, herbicides or toxic substances are used during any cultivation or manufacturing step of the product. There are no carcinogens or compounds that may degraded or metabolized to carcinogens used in the manufacturing process or known within *Haematococcus* algae.

Safety Studies of *Haematococcus* Algae Meal

Acute oral toxicity studies have been conducted on Charles River CD rats. The dosage level was 5,000 mg/kg and was administered as a 0.5% aqueous methylcellulose solution. Each lot was administered to separate groups of 10 rats that consisted of five males and five females. Groups for each treatment effect were evaluated for mortality, pharmacotoxic signs, body weights, and necropsy examinations during the 13-day study.

The results demonstrated that the LD_{50} value of each lot was greater than the administered dose of 5,000 mg/kg. No visible abnormalities were observed, nor differences in body weights during the study. The postmortem examination did not reveal any abnormalities in rats sacrificed at the end of the study.

Additional acute oral toxicity studies were conducted with both male and female mice. *Haematococcus* algae meal was suspended in distilled water for injection to give a 30% solution (w/v). The solution was forced by oral administration once using a gastric probe. The dosages ranged from 10,417-18,000 mg/kg, no mortalities were observed. The postmortem examination did not reveal any abnormalities in the rats that were sacrificed at the end of the study. The oral LD_{50} was judged to be 18,000 mg/kg or above.

A mutagenicity test using *Salmonella typhimurium* strain TA100, TA1535, TA98, TA1537, TA1538 and *E. coli* WP2 uvr A. A sample of *Haematococcus* algae meal was formulated into a 50 mg/ml solution of dimethyl sulfoxide. The formulation was spread onto the test petri plates in the presence of the microbial cultures with positive controls. The positive controls 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 1-ethyl-2-nitro-3-nitrosoguanidine, 9-aminoacridine, 2-aminoanthracene, and 2-nitrofluorene showed a remarkable increase in the number of reverent colonies compared with the solvent control.

In contrast to these results, the *Haematococcus* algae meal sample showed no significant increase in the number of reverent colonies in every case compared to the solvent control. This demonstrated that the mutagenicity of the sample under the employed conditions were negative.

Fish tissues from a *Haematococcus* algae feeding study of rainbow trout were analyzed for toxic effects and neoplasia. All tissues examined were normal in appearance with no indication of disease, toxicity or neoplasia. All fish examined were in excellent nutritional status with abundant body fat. Gross findings indicate that no adverse effects on health were observed from *Haematococcus* algae meal as the dietary source of astaxanthin.

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NatuRose™ Technical Bulletin #060
Revision Date: March 18, 1999

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Figure 1 (NatuRose Technical Bulletin): Isomers of Astaxanthin

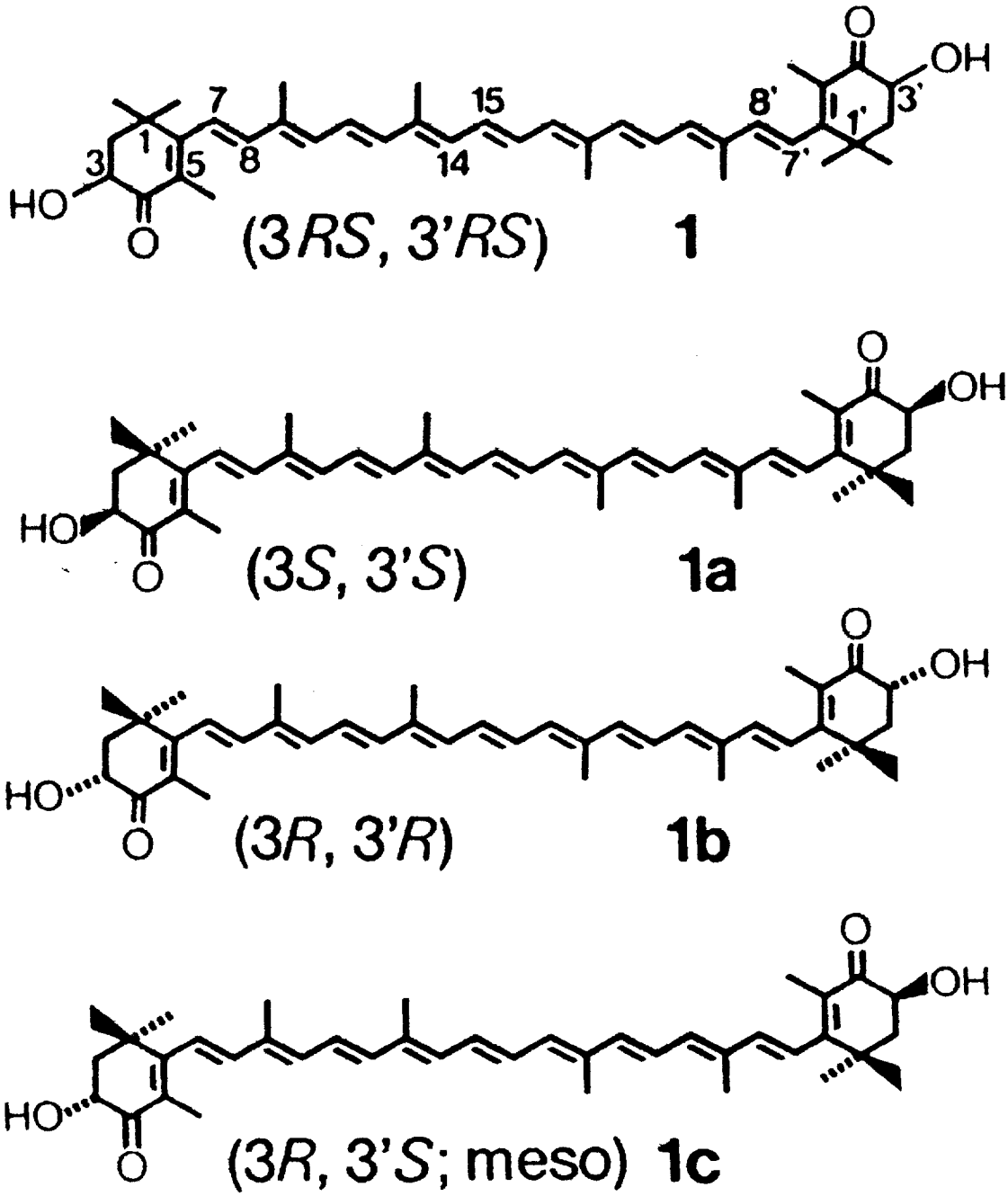


Figure 2 NatuRose Technical Bulletin): Astaxanthin pathway of *Haematococcus*

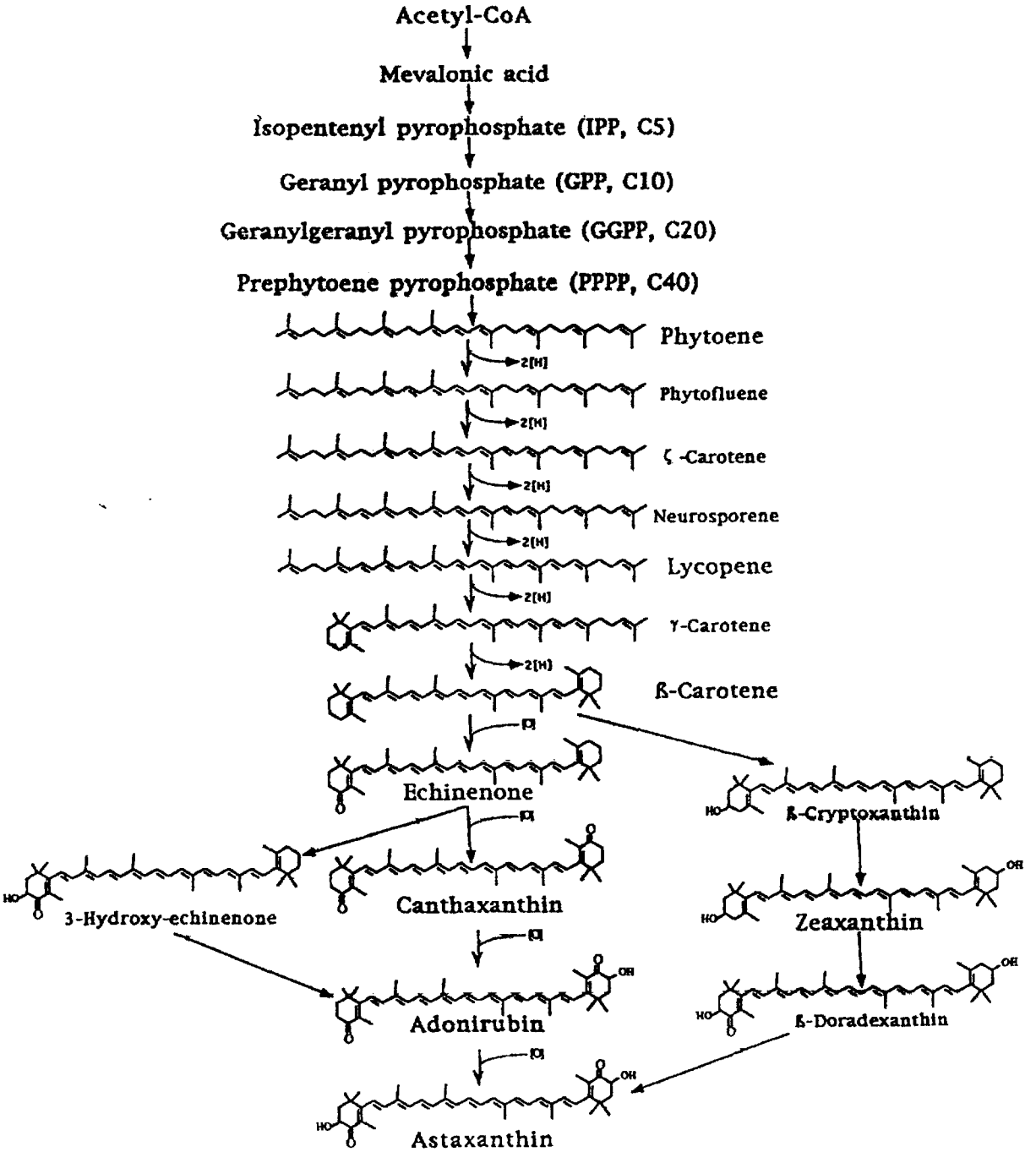
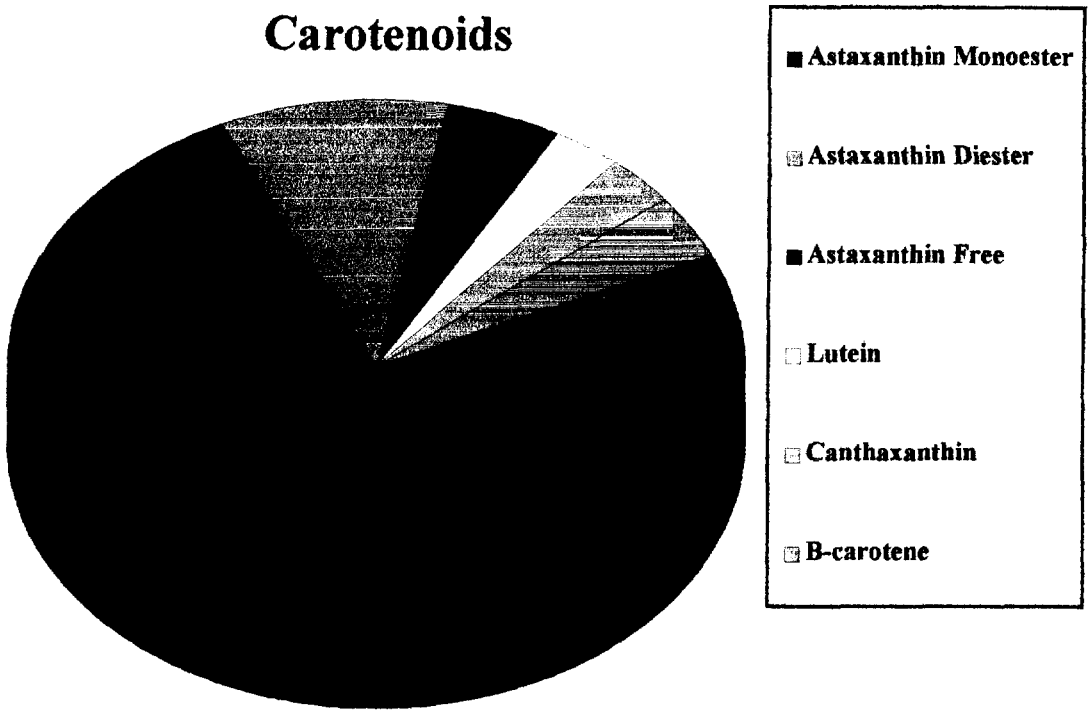


Figure 3: ***NatuRose*-Natural Astaxanthin Carotenoids**



- The carotenoid composition of *NatuRose* is similar to that of krill, shrimp, and crawfish.
- Esterified astaxanthin is inherently more stable to heat and oxygen.
- Canthaxanthin and β-carotene are converted to astaxanthin by shrimp and Koi species.

Haematococcus Algae Safety Summary

I. Safety Investigations

1. Possible contaminants

Three manufactured lots of *Haematococcus* algae were assayed for the following possible contaminants listed in Table 1. All tests were below the range of detectability or negative (Appendices 1, 2, and 3).

Table 1

Chlorinated hydrocarbon pesticides:	
Aldrin	ND <5.0 ppb
Lindane	ND <5.0 ppb
DDE	ND <10.0 ppb
DDD	ND <10.0 ppb
DDT	ND <10.0 ppb
Dieldrin	ND <5.0 ppb
Endrin	ND <10.0 ppb
Heptachlor	ND <5.0 ppb
Heptachlor Epoxide	ND <5.0 ppb
Methoxychlor	ND <2.0 ppb
Polychlorinated Biphenyls:	
PCB	ND <10.0 ppb
USP XXI Heavy metals:	< 1.25 ppm
Aflatoxin:	ND <1 ppb
Pathogens:	
Pseudomonas aeruginosa	Negative 10 g.
Salmonella	Negative 10 g.
E. coli	Negative 10 g.
Coagulase positive *Staphylococcus*	Negative 10 g.

ND=Not Detected

2. Heavy metal analysis

Five lots of NatuRose *Haematococcus* algae were analyzed for heavy metals and the results of the study are listed in Table 2 below. The raw data is attached as Appendix 8.

Table 2

	AX10176	AX04227	AX12106	AX05207	AX05317
arsenic	0.45 ppm	<3 ppm	<0.5 ppm	0.32 ppm	0.62 ppm
cadmium	<0.5 ppm	<0.5 ppm	<0.5 ppm	<0.5 ppm	<0.5 ppm
lead	4.0 ppm	<1 ppm	1.2 ppm	<1 ppm	<1 ppm
mercury	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm	<0.25 ppm

3. Acute oral toxicity study of *Haematococcus* algae in rats

Three different lots of *Haematococcus* algae were administered once orally via gavage to separate groups of Charles River CD rats. The dosage level was 5,000 mg/kg and was administered as a 0.5% aqueous methylcellulose solution. Each lot was administered to separate groups of 10 rats, which consisted of five males and five females. Groups for each treatment effect were evaluated for mortality, pharmacotoxic signs, body weights, and necropsy examinations during the 13-day study.

The results demonstrated that the LD50 value of each lot was greater than the administered dose of 5,000 mg/kg. No visible abnormalities were observed, nor differences in body weights during the study. The postmortem examination did not reveal any abnormalities in rats sacrificed at the end of the study (Appendix 23).

4. Acute oral toxicity study of *Haematococcus* algae in mice

One lot of *Haematococcus* algae was administered orally to 20 male and female mice. A 30 g. sample of *Haematococcus* algae was suspended in distilled water for injection to give a 30% solution (w/v). The solution was forced by oral administration once using a gastric probe. The dosages ranged from 10,417-18,000 mg/kg, no mortalities were observed. The postmortem examination did not reveal any abnormalities in the rats that were sacrificed at the end of the study. The oral LD_{50} was judged to be 18,000 mg/kg or above (Appendix 24).

5. Mutagenicity Test of *Haematococcus* algae

A mutagenicity test using *Salmonella typhimurium* strain TA100, TA1535, TA98, TA1537, TA1538 and *E. coli* WP2 uvr A. A sample of *Haematococcus* algae was formulated into a 50 mg/ml solution of dimethyl sulfoxide. The formulation was spread onto the test petri plates in the presence of the microbial cultures with positive controls. The positive controls 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide, 1-ethyl-2-nitro-3-nitrosoguanidine, 9-aminoacridine, 2-aminoanthracene, and 2-nitrofluorene showed a remarkable increase in the number of revertant colonies compared with the solvent control. In contrast to these results, the *Haematococcus* algae sample showed no significant increase in the number of revertant colonies in every case compared to the solvent control. This demonstrates that the mutagenicity of the sample under the employed conditions were negative (Appendix 25).

6. Pathological study of rainbow trout fed *Haematococcus* algae

Fish tissues from the rainbow trout feeding study (Appendix 16) were analyzed for toxic effects and neoplasia. All tissues examined were normal in appearance with no indication of disease, toxicity or neoplasia. All fish examined were in excellent nutritional status with abundant body fat. Gross findings indicate that no adverse effects on health were observed from *Haematococcus* algae as the dietary source of astaxanthin (Appendix 26).

7. Known toxicity of *Haematococcus* algae

Haematococcus algae has never been associated with any toxicity in the literature.

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Haematococcus CAP

APPENDIX 1

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1139 E. DOMINGUEZ ST. SUITE I
CARSON, CALIFORNIA 90746
(213) 637-7121

REPORT # 75220

SAMPLE DESCRIPTION - Sample # A-104

PESTICIDES
(CHLORINATED HYDROCARBONS)

Includes:

Aldrin	N.D. <5.0 ppb
Lindane	N.D. <5.0 ppb
DDE	N.D. <10.0 ppb
DDD	N.D. <10.0 ppb
DDT	N.D. <10.0 ppb
Dieldrin	N.D. <5.0 ppb
Endrin	N.D. <10.0 ppb
Heptachlor	N.D. <5.0 ppb
Heptachlor Epoxide	N.D. <5.0 ppb
Methoxychlor	N.D. <2.0 ppb

POLYCHLORINATED BIPHENYLS
(PCB) N.D. <10.0 ppb

USP XXI HEAVY METALS	AFLATOXIN	PSEUDOMONAS AERUGINOSA
<1.0 ppm	ND <1 ppb	Negative 10g

* SALMONELLA	* E. COLI	* COAGULASE POSITIVE STAPHYLOCOCCUS
Negative 10g	Negative 10g	Negative 10g

N.D. indicates None Detected.

* Microbiological methods provided by Bacteriological Analytical Manual, 6th Edition, 1984, U.S. Food and Drug Administration.

Haematococcus CAP

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APPENDIX 2

Silliker Laboratories

ANALYTICAL • MICROBIOLOGY • CHEMISTRY • CONSULTING

1139 E. DOMINGUEZ ST. SUITE I
CARSON, CALIFORNIA 90746
(213) 637-7121

REPORT # 75220

SAMPLE DESCRIPTION - Sample # IFL03E

PESTICIDES
(CHLORINATED HYDROCARBONS)

Includes:

Aldrin	N.D. <5.0 ppb
Lindane	N.D. <5.0 ppb
DDE	N.D. <10.0 ppb
DDD	N.D. <10.0 ppb
DDT	N.D. <10.0 ppb
Dieldrin	N.D. <5.0 ppb
Endrin	N.D. <10.0 ppb
Heptachlor	N.D. <5.0 ppb
Heptachlor Epoxide	N.D. <5.0 ppb
Methoxychlor	N.D. <2.0 ppb

POLYCHLORINATED BIPHENYLS
(PCB) N.D. <10.0 ppb

USP XXI HEAVY METALS	AFLATOXIN	PSEUDOMONAS AERUGINOSA
1.22 ppm	ND <1 ppb	Negative 10g

* SALMONELLA	* E. COLI	* COAGULASE POSITIVE STAPHYLOCOCCUS
Negative 10g	Negative 10g	Negative 10g

N.D. indicates None Detected.

* Microbiological methods provided by Bacteriological Analytical Manual, 6th Edition, 1984, U.S. Food and Drug Administration.

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Haematococcus CAP

APPENDIX 3

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(213) 637-7121

REPORT # 75220

SAMPLE DESCRIPTION - Sample # DA01001

PESTICIDES
(CHLORINATED HYDROCARBONS)

Includes:

Aldrin	N.D. <5.0 ppb
Lindane	N.D. <5.0 ppb
DDE	N.D. <10.0 ppb
DDD	N.D. <10.0 ppb
DDT	N.D. <10.0 ppb
Dieldrin	N.D. <5.0 ppb
Endrin	N.D. <10.0 ppb
Heptachlor	N.D. <5.0 ppb
Heptachlor Epoxide	N.D. <5.0 ppb
Methoxychlor	N.D. <2.0 ppb

POLYCHLORINATED BIPHENYLS
(PCB) N.D. <10.0 ppb

USP XXI HEAVY METALS	AFLATOXIN	PSEUDOMONAS AERUGINOSA
<1.0 ppm	ND <1 ppb	Negative 10g

* SALMONELLA	* E. COLI	* COAGULASE POSITIVE STAPHYLOCOCCUS
Negative 10g	Negative 10g	Negative 10g

N.D. indicates None Detected.

* Microbiological methods provided by Bacteriological Analytical Manual, 6th Edition, 1984, U.S. Food and Drug Administration.

Appendix 8

Woodson-Tenent Laboratories, Inc.

345 ADAMS AVE
P O BOX 2135
MEMPHIS TN 38101
(901)521-4500

W-T SAMPLE NO.: M96-632529
SAMPLE OF: AX SPRAY-DRIED POWDER
SAMPLE ID: AX 10176
PO NUMBER: 055253
CUST #: 01274500

W-T REPORTING DATE: 11/11/96
W-T ENTRY DATE: 10/21/96

CYANOTECH CORPORATION
ATTN SHANE ROHAN
73-4460 QUEEN KAAHAMANU
KAILUA-KONA HI 96740

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
AMINO ACID PROFILE	LISTED BELOW		
TRYPTOPHAN	0.32	%	
ASPARTIC ACID	1.87	%	
THREONINE	1.02	%	
SERINE	0.94	%	
GLUTAMIC ACID	2.39	%	
PROLINE	0.86	%	
GLYCINE	1.17	%	
ALANINE	1.74	%	
CYSTINE	0.21	%	
VALINE	1.10	%	
METHIONINE	0.38	%	
ISOLEUCINE	0.73	%	
LEUCINE	1.66	%	
TYROSINE	0.48	%	
PHENYLALANINE	0.88	%	
HISTIDINE	0.76	%	
LYSINE, TOTAL	1.16	%	
ARGININE	0.81	%	
FATTY ACID PROFILE, % BY WEIGHT	LISTED BELOW		
C14:0 TETRADECANOIC (MYRISTIC)	0.10	%	
C16:0 HEXADECANOIC (PALMITIC)	6.15	%	
C16:1 HEXADECENOIC (PALMITOLEIC)	0.05	%	
C17:0 HEPTADECANOIC (MARGARIC)	0.03	%	
C17:1 HEPTADECENOIC MARGAROLEIC	0.23	%	
C18:0 OCTADECANOIC (STEARIC)	0.46	%	
C18:1 OCTADECENOIC (OLEIC)	5.31	%	
C18:2 OCTADECADIENOIC (LINOLEIC)	4.40	%	
C18:3 OCTADECATRIENOIC (LINOLENIC)	2.11	%	

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M96-632529
SAMPLE OF: AX SPRAY-DRIED POWDER
SAMPLE ID: AX 10176
PO NUMBER: 055253
CUST #: 01274500

W-T REPORTING DATE: 11/11/96
W-T ENTRY DATE: 10/21/96

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
C18:3 GAMMA LINOLENIC OMEGA 6	0.29	%	
C18:4 OCTADECATETRAENOIC	0.25	%	
C20:0 EICOSANOIC (ARACHIDIC)	0.12	%	
C20:1 EICOSENOIC (GADOLEIC)	0.05	%	
C20:2 EICOSADIENOIC	0.21	%	
C20:3 EICOSATRIENOIC GAMMA	0.08	%	
C20:4 EICOSATETRAENOIC (ARACHIDONIC	0.22	%	
C20:5 EICOSAPENTAENOIC OMEGA 3	0.08	%	
C22:0 DOCOSANOIC (BEHENIC)	0.08	%	
C24:0 TETRACOSANOIC (LIGNOCERIC)	0.05	%	
PROTEIN - KJELDAHL	25.11	%	
ASH	11.07	%	
CARBOHYDRATES, CALCULATED	37.1	%	
CALCIUM	0.98	%	
IRON	0.82	%	
MAGNESIUM	0.85	%	
TOTAL FAT	21.22	%	
SATURATED FATTY ACIDS	7.00	%	
MOISTURE BY VACUUM OVEN	5.51	%	
BIOTIN	0.108	MG/100G	
FOLIC ACID	0.253	MG/100G	
NIACIN	7.51	MG/100G	
PANTOTHENIC ACID	2.33	MG/100G	
VITAMIN B1 - THIAMINE HYDROCHLORIDE	1.06	MG/100G	
VITAMIN B2 - RIBOFLAVIN	1.83	MG/100G	
VITAMIN B6	0.238	MG/100G	
VITAMIN B12	80.4	MCG/100G	
VITAMIN C - ASCORBIC ACID	12.5	MG/100G	
VITAMIN E (LOW LEVEL)	58.4	IU/100G	

Woodson-Tenent Laboratories, Inc.

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(901)521-4500

W-T SAMPLE NO.: M96-632529
SAMPLE OF: AX SPRAY-DRIED POWDER
SAMPLE ID: AX 10176
PO NUMBER: 055253
CUST #: 01274500

W-T REPORTING DATE: 11/15/96
W-T ENTRY DATE: 10/21/96

CYANOTECH CORPORATION
ATTN SHANE ROHAN
73-4460 QUEEN KAAHAMANU
KAILUA-KONA HI 96740

REPORT OF ANALYSIS
PARTIAL REPORT

TEST	RESULT	UNITS
FATTY ACID PROFILE, % BY WEIGHT	LISTED BELOW	
C14:0 TETRADECANOIC (MYRISTIC)	0.10	%
C16:0 HEXADECANOIC (PALMITIC)	6.15	%
C16:1 HEXADECENOIC (PALMITOLEIC)	0.05	%
C17:0 HEPTADECANOIC (MARGARIC)	0.03	%
C17:1 HEPTADECENOIC MARGAROLEIC	0.23	%
C18:0 OCTADECANOIC (STEARIC)	0.46	%
C18:1 OCTADECENOIC (OLEIC)	5.31	%
C18:2 OCTADECADIENOIC (LINOLEIC)	4.40	%
C18:3 OCTADECATRIENOIC (LINOLENIC)	2.11	%
C18:3 GAMMA LINOLENIC OMEGA 6	0.29	%
C18:4 OCTADECATETRAENOIC	0.25	%
C20:0 EICOSANOIC (ARACHIDIC)	0.12	%
C20:1 EICOSENOIC (GADOLEIC)	0.05	%
C20:2 EICOSADIENOIC	0.21	%
C20:3 EICOSATRIENOIC GAMMA	0.08	%
C20:4 EICOSATETRAENOIC (ARACHIDONIC	0.22	%
C20:5 EICOSAPENTAENOIC OMEGA 3	0.08	%
C22:0 DOCOSANOIC (BEHENIC)	0.08	%
C24:0 TETRACOSANOIC (LIGNOCERIC)	0.05	%
PROTEIN - KJELDAHL	25.11	%
ASH	11.07	%
CARBOHYDRATES, CALCULATED	37.1	%
CALCIUM	0.98	%
IRON	0.82	%
MAGNESIUM	0.85	%
TOTAL FAT	21.22	%

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W-T SAMPLE NO.: M97-719073
SAMPLE OF: RED ALGAE
SAMPLE ID: LOT AX10176
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 6/16/97
W-T ENTRY DATE: 6/06/97

CYANOTECH CORPORATION
ATTN SHANE ROHAN
73-4460 QUEEN KAAHAMANU
KAILUA-KONA HI 96740

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
ARSENIC	0.45	PPM	
CADMIUM	<0.5	PPM	
LEAD	4.0	PPM	
MERCURY	<0.25	PPM	
NICKEL	0.024	%	

RESPECTFULLY SUBMITTED,
WOODSON-TENENT LABORATORIES, INC.

J A WILLIAMS
BRANCH MANAGER

Woodson-Tenent Laboratories, Inc.

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W-T SAMPLE NO.: M96-632529
SAMPLE OF: AX SPRAY-DRIED POWDER
SAMPLE ID: AX 10176
PO NUMBER: 055253
CUST #: 01274500

W-T REPORTING DATE: 11/11/96
W-T ENTRY DATE: 10/21/96

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73-4460 QUEEN KAAHAMANU
KAILUA-KONA HI 96740

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
PROTEIN - KJELDAHL	25.11	%	
ASH	11.07	%	
CARBOHYDRATES, CALCULATED	37.1	%	
CALCIUM	0.98	%	
IRON	0.82	%	
MAGNESIUM	0.85	%	
TOTAL FAT	21.22	%	
SATURATED FATTY ACIDS	7.00	%	
MOISTURE BY VACUUM OVEN	5.51	%	
BIOTIN	0.108	MG/100G	
FOLIC ACID	0.253	MG/100G	
NIACIN	7.51	MG/100G	
PANTOTHENIC ACID	2.33	MG/100G	
VITAMIN B1 - THIAMINE HYDROCHLORIDE	1.06	MG/100G	
VITAMIN B2 - RIBOFLAVIN	1.83	MG/100G	
VITAMIN B6	0.238	MG/100G	
VITAMIN B12	80.4	MCG/100G	
VITAMIN C - ASCORBIC ACID	12.5	MG/100G	
VITAMIN E (LOW LEVEL)	58.4	IU/100G	

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J A WILLIAMS
BRANCH MANAGER

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W-T SAMPLE NO.: M 96-632529
SAMPLE OF: AX SPRAY-DRIED POWDER
SAMPLE ID: AX 10176
PO NUMBER: 055253
CUST #: 01274500

W-T REPORTING DATE: 11/15/96
W-T ENTRY DATE: 10/21/96

REPORT OF ANALYSIS
PARTIAL REPORT

TEST	RESULT	UNITS
SATURATED FATTY ACIDS	7.00	%
MOISTURE BY VACUUM OVEN	5.51	%
BIOTIN	0.108	MG/100G
FOLIC ACID	0.253	MG/100G
NIACIN	7.51	MG/100G
PANTOTHENIC ACID	2.33	MG/100G
VITAMIN B1 - THIAMINE HYDROCHLORIDE	1.06	MG/100G
VITAMIN B2 - RIBOFLAVIN	1.83	MG/100G
VITAMIN B6	0.238	MG/100G
VITAMIN B12	80.4	MCG/100G
VITAMIN C - ASCORBIC ACID	12.5	MG/100G
VITAMIN E (LOW LEVEL)	58.4	IU/100G

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345 ADAMS AVE
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(901)521-4500 Shelly Barbour

W-T SAMPLE NO.: M97-704010
SAMPLE OF: NOT STATED
SAMPLE ID: SPRAY DRIED AX LOT# AX12106
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 3/06/97
W-T ENTRY DATE: 2/05/97

CYANOTECH CORPORATION
ATTN SHANE ROHAN
73-4460 QUEEN KAAHAMANU
KAILUA-KONA HI 96740

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
ARSENIC	<0.5	PPM	
CADMIUM	<0.5	MCG/G	
CALCIUM	1.2	%	
IRON	0.14	%	
LEAD	1.2	MCG/G	
MAGNESIUM	1.4	%	
MERCURY	<0.25	MCG/G	
NICKEL	0.038	%	
PHOSPHORUS	0.96	%	
POTASSIUM	0.45	%	
SODIUM	0.52	%	
SULFUR	0.22	%	
AMINO ACID PROFILE	LISTED BELOW		
TRYPTOPHAN	0.56	%	
ASPARTIC ACID	1.98	%	
THREONINE	1.05	%	
SERINE	0.96	%	
GLUTAMIC ACID	2.26	%	
PROLINE	1.00	%	
GLYCINE	1.28	%	
ALANINE	1.87	%	
CYSTINE	0.18	%	
VALINE	1.25	%	
METHIONINE	0.44	%	
ISOLEUCINE	0.87	%	
LEUCINE	1.82	%	
TYROSINE	0.60	%	
PHENYLALANINE	0.99	%	
HISTIDINE	0.76	%	

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345 ADAMS AVE
P O BOX 2135
MEMPHIS TN 38101
(901)521-4500

W-T SAMPLE NO.: M97-704010
SAMPLE OF: NOT STATED
SAMPLE ID: SPRAY DRIED AX LOT# AX12106
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 3/06/97
W-T ENTRY DATE: 2/05/97

REPRINT DATE: 3/10/97

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73-4460 QUEEN KAAHAMANU
KAILUA-KONA HI 96740

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS
ARSENIC	<0.5	PPM
CADMIUM	<0.5	MCG/G
CALCIUM	1.2	%
IRON	0.14	%
LEAD	1.2	MCG/G
MAGNESIUM	1.4	%
MERCURY	<0.25	MCG/G
NICKEL	0.038	%
PHOSPHORUS	0.96	%
POTASSIUM	0.45	%
SODIUM	0.52	%
SULFUR	0.22	%
AMINO ACID PROFILE	LISTED BELOW	
TRYPTOPHAN	0.56	%
ASPARTIC ACID	1.98	%
THREONINE	1.05	%
SERINE	0.96	%
GLUTAMIC ACID	2.26	%
PROLINE	1.00	%
GLYCINE	1.28	%
ALANINE	1.87	%
CYSTINE	0.18	%
VALINE	1.25	%
METHIONINE	0.44	%
ISOLEUCINE	0.87	%
LEUCINE	1.82	%
TYROSINE	0.60	%

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M97-704010
SAMPLE OF: NOT STATED
SAMPLE ID: SPRAY DRIED AX LOT# AX12106
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 3/06/97
W-T ENTRY DATE: 2/05/97

REPORT OF ANALYSIS

TEST	RESULT	UNITS	LAB CODE #
LYSINE, TOTAL	1.22	%	
ARGININE	1.10	%	
BIOTIN	0.665	MG/LB	
FOLIC ACID	1.72	MG/LB	
NIACIN	34.7	MG/LB	
PANTOTHENIC ACID	9.38	MG/LB	
VITAMIN B1 - THIAMINE HYDROCHLORIDE	1.61	MG/LB	
VITAMIN B2 - RIBOFLAVIN	9.36	MG/LB	
VITAMIN B6	1.18	MG/LB	
VITAMIN B12	0.454	MG/LB	
VITAMIN C - ASCORBIC ACID	82.7	MG/LB	
VITAMIN E (LOW LEVEL)	214	IU/LB	
PROTEIN - KJELDAHL	25.10	%	
ASH	22.62	%	
CARBOHYDRATES, CALCULATED	40.00	%	
TOTAL FAT	7.143	%	
C12:0 DODECANOIC (LAURIC)	<0.005	%	
C14:0 TETRADECANOIC (MYRISTIC)	0.039	%	
C15:0 PENTADECANOIC	0.005	%	
C16:0 HEXADECANOIC (PALMITIC)	2.068	%	
C16:1 HEXADECENOIC (PALMITOLEIC)	0.020	%	
C17:0 HEPTADECANOIC (MARGARIC)	0.013	%	
C17:1 HEPTADECENOIC MARGAROLEIC	0.095	%	
C18:0 OCTADECANOIC (STEARIC)	0.142	%	
C18:1 OCTADECENOIC (OLEIC)	1.665	%	
C18:2 OCTADECADIENOIC (LINOLEIC)	1.445	%	
C18:3 OCTADECATRIENOIC (LINOLENIC)	0.860	%	
C18:3 GAMMA LINOLENIC OMEGA 6	0.090	%	
C18:4 OCTADECATETRAENOIC	0.097	%	
C20:0 EICOSANOIC (ARACHIDIC)	0.040	%	
C20:1 EICOSENOIC (GADOLEIC)	0.015	%	
C20:2 EICOSADIENOIC	0.058	%	
C20:3 EICOSATRIENOIC	<0.005	%	
C20:3 EICOSATRIENOIC GAMMA	0.023	%	
C20:4 EICOSATETRAENOIC (ARACHIDONIC	0.082	%	
C20:5 EICOSAPENTAENOIC OMEGA 3	0.031	%	
C22:0 DOCOSANOIC (BEHENIC)	0.022	%	
C24:0 TETRACOSANOIC (LIGNOCERIC)	0.013	%	

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M 97-704010
SAMPLE OF: NOT STATED
SAMPLE ID: SPRAY DRIED AX LOT# AX12106
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 3/06/97
W-T ENTRY DATE: 2/05/97

REPRINT DATE: 3/10/97

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS
PHENYLALANINE	0.99	%
HISTIDINE	0.76	%
LYSINE, TOTAL	1.22	%
ARGININE	1.10	%
BIOTIN	0.665	MG/LB
FOLIC ACID	1.72	MG/LB
NIACIN	34.7	MG/LB
PANTOTHENIC ACID	9.38	MG/LB
VITAMIN B1 - THIAMINE HYDROCHLORIDE	1.61	MG/LB
VITAMIN B2 - RIBOFLAVIN	9.36	MG/LB
VITAMIN B6	1.18	MG/LB
VITAMIN B12	0.454	MG/LB
VITAMIN C - ASCORBIC ACID	82.7	MG/LB
VITAMIN E (LOW LEVEL)	214	IU/LB
PROTEIN - KJELDAHL	25.10	%
ASH	22.62	%
CARBOHYDRATES, CALCULATED	40.00	%
TOTAL FAT	7.143	%
C12:0 DODECANOIC (LAURIC)	<0.005	%
C14:0 TETRADECANOIC (MYRISTIC)	0.039	%
C15:0 PENTADECANOIC	0.005	%
C16:0 HEXADECANOIC (PALMITIC)	2.068	%
C16:1 HEXADECENOIC (PALMITOLEIC)	0.020	%
C17:0 HEPTADECANOIC (MARGARIC)	0.013	%
C17:1 HEPTADECENOIC MARGAROLEIC	0.095	%
C18:0 OCTADECANOIC (STEARIC)	0.142	%
C18:1 OCTADECENOIC (OLEIC)	1.665	%
C18:2 OCTADECADIENOIC (LINOLEIC)	1.445	%
C18:3 OCTADECATRIENOIC (LINOLENIC)	0.860	%
C18:3 GAMMA LINOLENIC OMEGA 6	0.090	%
C18:4 OCTADECATETRAENOIC	0.097	%
C20:0 EICOSANOIC (ARACHIDIC)	0.040	%
C20:1 EICOSENOIC (GADOLEIC)	0.015	%
C20:2 EICOSADIENOIC	0.058	%

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M 97-704010
SAMPLE OF: NOT STATED
SAMPLE ID: SPRAY DRIED AX LOT# AX12106
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 3/06/97
W-T ENTRY DATE: 2/05/97

REPRINT DATE: 3/10/97

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS
C20:3 EICOSATRIENOIC	<0.005	%
C20:3 EICOSATRIENOIC GAMMA	0.023	%
C20:4 EICOSATETRAENOIC (ARACHIDONIC	0.082	%
C20:5 EICOSAPENTAENOIC OMEGA 3	0.031	%
C22:0 DOCOSANOIC (BEHENIC)	0.022	%
C24:0 TETRACOSANOIC (LIGNOCERIC)	0.013	%
SATURATED FATTY ACIDS	2.344	%
MOISTURE BY VACUUM OVEN	5.13	%

RESPECTFULLY SUBMITTED,
WOODSON-TENENT LABORATORIES, INC.

J A WILLIAMS
BRANCH MANAGER

Woodson-Tenent Laboratories, Inc.

345 ADAMS AVE
P O BOX 2135
MEMPHIS TN 38101
(901)521-4500

W-T SAMPLE NO.: M97-714526
SAMPLE OF: AX SPRAY DRIED POWDER
SAMPLE ID: LOT# AXM04227
PO NUMBER: 56080
CUST #: 01274500

W-T REPORTING DATE: 5/20/97
W-T ENTRY DATE: 4/25/97

CYANOTECH CORPORATION
ATTN SHANE ROHAN
73-4460 QUEEN KAAHAMANU
KAILUA-KONA HI 96740

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
ARSENIC	<3	PPM	
CADMIUM	<0.5	MCG/G	
CALCIUM	3.3	%	
IRON	1.0	%	
LEAD	<1	MCG/G	
MANGANESE	0.020	%	
MERCURY	<0.25	MCG/G	
NICKEL	0.018	%	
PHOSPHORUS	0.83	%	
POTASSIUM	0.40	%	
SODIUM	0.49	%	
SULFUR	0.19	%	
AMINO ACID PROFILE	LISTED BELOW		
TRYPTOPHAN	0.05	%	
ASPARTIC ACID	1.37	%	
THREONINE	0.78	%	
SERINE	0.73	%	
GLUTAMIC ACID	1.70	%	
PROLINE	0.69	%	
GLYCINE	0.84	%	
ALANINE	1.30	%	
CYSTINE	0.16	%	
VALINE	0.83	%	
METHIONINE	0.32	%	
ISOLEUCINE	0.55	%	
LEUCINE	1.21	%	
TYROSINE	0.40	%	
PHENYLALANINE	0.61	%	
HISTIDINE	0.52	%	

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M97-714526
SAMPLE OF: AX SPRAY DRIED POWDER
SAMPLE ID: LOT# AXM04227
PO NUMBER: 56080
CUST #: 01274500

W-T REPORTING DATE: 5/20/97
W-T ENTRY DATE: 4/25/97

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
LYSINE, TOTAL	0.75	%	
ARGININE	0.86	%	
BIOTIN	0.180	MG/LB	
FOLIC ACID	1.23	MG/LB	
NIACIN	20.2	MG/LB	
PANTOTHENIC ACID	2.80	MG/LB	
VITAMIN B1 - THIAMINE HYDROCHLORIDE	0.0863	MG/LB	
VITAMIN B2 - RIBOFLAVIN	5.17	MG/LB	
VITAMIN B6	4.50	MG/LB	
VITAMIN B12	0.467	MG/LB	
VITAMIN C - ASCORBIC ACID	6.42	MG/LB	
VITAMIN E (LOW LEVEL)	92.0	IU/LB	
PROTEIN - KJELDAHL	17.30	%	
ASH	24.47	%	
CARBOHYDRATES, CALCULATED	36.9	%	
TOTAL FAT + SAT FATTY ACIDS + FAC	SEE BELOW		
TOTAL FAT	13.94	%	
C12:0 DODECANOIC (LAURIC)	<0.01	%	
C14:0 TETRADECANOIC (MYRISTIC)	0.07	%	
C15:0 PENTADECANOIC	0.01	%	
C16:0 HEXADECANOIC (PALMITIC)	3.76	%	
C16:1 HEXADECENOIC (PALMITOLEIC)	0.07	%	
C17:0 HEPTADECANOIC (MARGARIC)	0.03	%	
C17:1 HEPTADECENOIC MARGAROLEIC	0.19	%	
C18:0 OCTADECANOIC (STEARIC)	0.22	%	
C18:1 OCTADECENOIC (OLEIC)	3.42	%	
C18:2 OCTADECADIENOIC (LINOLEIC)	3.07	%	
C18:3 OCTADECATRIENOIC (LINOLENIC)	1.44	%	
C18:3 GAMMA LINOLENIC OMEGA 6	0.21	%	
C18:4 OCTADECATETRAENOIC	0.19	%	
C20:0 EICOSANOIC (ARACHIDIC)	0.07	%	
C20:1 EICOSENOIC (GADOLEIC)	0.03	%	
C20:2 EICOSADIENOIC	0.19	%	
C20:3 EICOSATRIENOIC	<0.01	%	
C20:3 EICOSATRIENOIC GAMMA	0.06	%	
C20:4 EICOSATETRAENOIC (ARACHIDONIC	0.15	%	
C20:5 EICOSAPENTAENOIC OMEGA 3	0.07	%	
C22:0 DOCOSANOIC (BEHENIC)	0.04	%	

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M97-714526
SAMPLE OF: AX SPRAY DRIED POWDER
SAMPLE ID: LOT# AXM04227
PO NUMBER: 56080
CUST #: 01274500

W-T REPORTING DATE: 5/20/97
W-T ENTRY DATE: 4/25/97

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
C24:0 TETRACOSANOIC (LIGNOCERIC)	0.03	%	
SATURATED FATTY ACIDS	4.23	%	
MOISTURE BY VACUUM OVEN	7.39	%	

RESPECTFULLY SUBMITTED,
WOODSON-TENENT LABORATORIES, INC.

J A WILLIAMS
BRANCH MANAGER

Woodson-Tenent Laboratories, Inc.

345 ADAMS AVE
P O BOX 2135
MEMPHIS TN 38101
(901)521-4500

W-T SAMPLE NO.: M97-719074
SAMPLE OF: RED ALGAE
SAMPLE ID: LOT AX05207
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 6/23/97
W-T ENTRY DATE: 6/06/97

CYANOTECH CORPORATION
ATTN SHANE ROHAN
73-4460 QUEEN KAAHAMANU
KAILUA-KONA HI 96740

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
PROTEIN - LECO	27.16	%	
ARSENIC	0.32	PPM	
CADMIUM	<0.5	PPM	
CALCIUM	<1	PPM	
IRON	0.86	%PM	
LEAD	<1	PPM	
MAGNESIUM	1.1	%	
MERCURY	<0.25	PPM	
NICKEL	0.011	%	
PHOSPHORUS	0.86	%	
POTASSIUM	0.33	%	
SODIUM	0.50	%	
SULFUR	0.31	%	
AMINO ACID PROFILE	LISTED BELOW		
TRYPTOPHAN	0.32	%	
ASPARTIC ACID	2.31	%	
THREONINE	1.24	%	
SERINE	1.06	%	
GLUTAMIC ACID	2.36	%	
PROLINE	0.93	%	
GLYCINE	1.32	%	
ALANINE	1.82	%	
CYSTINE	0.20	%	
VALINE	1.36	%	
METHIONINE	0.43	%	
ISOLEUCINE	0.97	%	
LEUCINE	1.82	%	
TYROSINE	0.63	%	
PHENYLALANINE	1.05	%	

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M97-719074
SAMPLE OF: RED ALGAE
SAMPLE ID: LOT AX05207
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 6/23/97
W-T ENTRY DATE: 6/06/97

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
HISTIDINE	0.55	%	
LYSINE, TOTAL	1.32	%	
ARGININE	1.34	%	
BIOTIN	0.350	MG/LB	
FOLIC ACID	0.936	MG/LB	
NIACIN	25.1	MG/LB	
PANTOTHENIC ACID	4.01	MG/LB	
VITAMIN B1 - THIAMINE HYDROCHLORIDE	<0.050	MG/LB	
VITAMIN B2 - RIBOFLAVIN	7.94	MG/LB	
VITAMIN B6	0.659	MG/LB	
VITAMIN B12	0.912	MG/LB	
VITAMIN C - ASCORBIC ACID	18.4	MG/LB	
VITAMIN E (LOW LEVEL)	233	IU/LB	
ASH	14.53	%	
TOTAL FAT + SAT FATTY ACIDS + FAC	LISTED BELOW		
TOTAL FAT	13.32	%	
C08:0 OCTANOIC (CAPRYLIC)	<0.01	%	
C12:0 DODECANOIC (LAURIC)	0.01	%	
C14:0 TETRADECANOIC (MYRISTIC)	0.09	%	
C15:0 PENTADECANOIC	0.02	%	
C16:0 HEXADECANOIC (PALMITIC)	3.55	%	
C16:1 HEXADECENOIC (PALMITOLEIC)	0.17	%	
C17:0 HEPTADECANOIC (MARGARIC)	0.03	%	
C17:1 HEPTADECENOIC MARGAROLEIC	0.19	%	
C18:0 OCTADECANOIC (STEARIC)	0.31	%	
C18:1 OCTADECENOIC (OLEIC)	3.32	%	
C18:2 OCTADECADIENOIC (LINOLEIC)	2.20	%	
C18:3 OCTADECATRIENOIC (LINOLENIC)	1.31	%	
C18:3 GAMMA LINOLENIC OMEGA 6	0.21	%	
C18:4 OCTADECATETRAENOIC	0.19	%	
C20:0 EICOSANOIC (ARACHIDIC)	0.10	%	
C20:1 EICOSENOIC (GADOLEIC)	0.08	%	
C20:2 EICOSADIENOIC	0.15	%	
C20:3 EICOSATRIENOIC	0.03	%	
C20:3 EICOSATRIENOIC GAMMA	0.09	%	
C20:4 EICOSATETRAENOIC (ARACHIDONIC	0.31	%	
C20:5 EICOSAPENTAENOIC OMEGA 3	0.18	%	
C22:0 DOCOSANOIC (BEHENIC)	0.05	%	

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M97-719074
SAMPLE OF: RED ALGAE
SAMPLE ID: LOT AX05207
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 6/23/97
W-T ENTRY DATE: 6/06/97

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
C22:1 DOCOSENOIC (ERUCIC)	0.04	%	
C22:2 DOCOSADIENOIC	0.02	%	
C22:4 DOCOSATETRAENOIC	0.02	%	
C22:5 DOCOSAPENTAENOIC	<0.01	%	
C22:6 DOCOSAHEXAENOIC OMEGA 3	0.01	%	
C24:0 TETRACOSANOIC (LIGNOCERIC)	0.04	%	
C24:1 TETRACOSENOIC (NERVONIC)	0.01	%	
SATURATED FATTY ACIDS	4.20	%	
MOISTURE BY VACUUM OVEN	5.73	%	

RESPECTFULLY SUBMITTED,
WOODSON-TENENT LABORATORIES, INC.

J A WILLIAMS
BRANCH MANAGER

Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M97-719075
SAMPLE OF: RED ALGAE
SAMPLE ID: LOT AX05317
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 6/23/97
W-T ENTRY DATE: 6/06/97

REPORT OF ANALYSIS

TEST	RESULT	UNITS	LAB CODE #
HISTIDINE	0.48	%	
LYSINE, TOTAL	1.19	%	
ARGININE	1.26	%	
BIOTIN	0.384	MG/LB	
FOLIC ACID	1.48	MG/LB	
NIACIN	35.2	MG/LB	
PANTOTHENIC ACID	3.95	MG/LB	
VITAMIN B1 - THIAMINE HYDROCHLORIDE	<0.050	MG/LB	
VITAMIN B2 - RIBOFLAVIN	7.59	MG/LB	
VITAMIN B6	0.744	MG/LB	
VITAMIN B12	0.530	MG/LB	
VITAMIN C - ASCORBIC ACID	30.1	MG/LB	
VITAMIN E (LOW LEVEL)	333	IU/LB	
ASH	15.87	%	
TOTAL FAT + SAT FATTY ACIDS + FAC	LISTED BELOW		
TOTAL FAT	13.37	%	
C12:0 DODECANOIC (LAURIC)	0.01	%	
C14:0 TETRADECANOIC (MYRISTIC)	0.07	%	
C15:0 PENTADECANOIC	<0.01	%	
C16:0 HEXADECANOIC (PALMITIC)	3.57	%	
C16:1 HEXADECENOIC (PALMITOLEIC)	0.09	%	
C17:0 HEPTADECANOIC (MARGARIC)	0.03	%	
C17:1 HEPTADECENOIC MARGAROLEIC	0.17	%	
C18:0 OCTADECANOIC (STEARIC)	0.23	%	
C18:1 OCTADECENOIC (OLEIC)	3.34	%	
C18:2 OCTADECADIENOIC (LINOLEIC)	2.56	%	
C18:3 OCTADECATRIENOIC (LINOLENIC)	1.62	%	
C18:3 GAMMA LINOLENIC OMEGA 6	0.25	%	
C18:4 OCTADECATETRAENOIC	0.22	%	
C20:0 EICOSANOIC (ARACHIDIC)	0.08	%	
C20:1 EICOSENOIC (GADOLEIC)	0.03	%	
C20:2 EICOSADIENOIC	0.18	%	
C20:3 EICOSATRIENOIC GAMMA	0.06	%	
C20:4 EICOSATETRAENOIC (ARACHIDONIC	0.14	%	
C20:5 EICOSAPENTAENOIC OMEGA 3	0.06	%	
C22:0 DOCOSANOIC (BEHENIC)	0.05	%	
C24:0 TETRACOSANOIC (LIGNOCERIC)	0.03	%	
SATURATED FATTY ACIDS	4.07	%	

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Woodson-Tenent Laboratories, Inc.

W-T SAMPLE NO.: M97-719075
SAMPLE OF: RED ALGAE
SAMPLE ID: LOT AX05317
PO NUMBER:
CUST #: 01274500

W-T REPORTING DATE: 6/23/97
W-T ENTRY DATE: 6/06/97

R E P O R T O F A N A L Y S I S

TEST	RESULT	UNITS	LAB CODE #
MOISTURE BY VACUUM OVEN	4.97	%	

RESPECTFULLY SUBMITTED,
WOODSON-TENENT LABORATORIES, INC.

J A WILLIAMS
BRANCH MANAGER

VITAMIN DIAGNOSTICS, Inc.

Telephone (908) 583-7773 ROUTE 35 AND INDUSTRIAL DRIVE • CLIFFWOOD BEACH, NEW JERSEY 07735

May 21, 1997

R. Todd Lorenze, Ph.D.
Scientific Director
Cyanotech Corporation
Hawaiian Ocean Science and
Technology Park
73-446 Queen Kaahumanu Hwy #102
Kailua-Kona, Hawaii 96740

Dear Dr. Lorenze:

Results of the carnitine analyses of the NatuRose materials you sent are as follows:

1) NatuRose 04307	Free carnitine = 4.0 ug/gm	Total carnitine = 7.3 ug/gm
2) NatuRose 03277	Free carnitine = 4.3 ug/gm	Total carnitine = 8.0 ug/gm
3) NatuRose 04289	Free carnitine = 2.9 ug/gm	Total carnitine = 7.0 ug/gm

If you have any questions or if we can be of any further assistance please do not hesitate to call or write.

Sincerely,

Susan Feingold
Laboratory Manager

Appendix 23

International Research
and Development Corporation
MATTAWAN, MICHIGAN, U.S.A. 49071 TELEPHONE (616) 668-3336

SPONSOR: Microbio Resources, Inc.

TEST ARTICLES: Algae Meal, Lot A-104
Algae Meal, Lot DA01001
Algae Meal, Lot 1FL03E

SUBJECT: Acute Oral Toxicity Study in Rats

DATE OF STUDY COMPLETION: May 19, 1989

QUALITY ASSURANCE STATEMENT

Study Title: Acute Oral Toxicity Study in Rats

Test Articles: Algae Meal, Lot A-104, Algae Meal, Lot DA01001, Algae Meal, Lot 1FL03E

This report has been reviewed by the International Research and Development Corporation Quality Assurance Department in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations of June 20, 1979 and as modified by the final rule effective October 5, 1987.

An inspection of the protocol for this study was conducted on February 8, 1989. A randomly sampled phase of the conduct of the study was inspected on January 26, 1989. Findings resulting from inspections, from a data audit, and from a review of the report were reported to management and the Study Director on January 30, 1989 and April 28, 1989.

Approved By: Margery J. Wirth 5/19/89

Margery J. Wirth, B.S. Date

Director of Quality Assurance

SYNOPSIS

The test articles, Algae Meal Lot Numbers A-104, DA01001 and 1FL03E, were administered once orally via gavage to separate groups of Charles River CD® rats as weight/volume suspensions in 0.5% aqueous methylcellulose. The dosage level was 5,000 mg/kg for each test article group. Each group consisted of five males and five females and were dosed at a volume of 20 ml/kg.

Criteria evaluted for treatment effect were mortality, pharmacotoxic signs, body weights and gross necropsy examinations.

Based on the results obtained, the LD_{50} value of each test article was estimated to be greater than the administered dose of 5,000 mg/kg. All rats survived to study termination. One male administered the test article lot number A-104 exhibited chromodacryorrhea within 1 hour post-dose on day 1. This sign persisted to day 3 and cleared by day 4. Four rats from the test article group lot number 1FL03E exhibited high carriage or decreased activity within 2 1/2 or 4 hours post-dose on day 1 and clearing by day 2. No visible abnormalities were observed in rats administered lot number DA01001.

There were no remarkable changes or differences observed in body weights during the study period and no visible abnormalities were observed in the rats sacrificed at study termination at the post-mortem examination.

STUDY SUMMARY

STUDY TITLE: Acute Oral Toxicity Study in Rats

STUDY INITIATED: 1/25/89

IRDC STUDY NUMBER: 538-002 DATE OF DOSING: 1/26/89

DATE OF NECROPSY: 2/09/89

TEST FACILITY: International Research and Development Corporation
Mattawan, Michigan 49071

SPONSOR: Microbio Resources, Inc.

TEST ARTICLES:

Sponsor Identification	IRDC Identification	Lot Number
Algae Meal	9902	A-104
Algae Meal	9903	DA01001
Algae Meal	9904	1FL03E

Storage Conditions: Sealed containers under refrigeration

OBJECTIVE: To evaluate the toxicity of the test articles after a single oral dose.

TEST SYSTEM:

Species: Rat Strain: Charles River CD®

Source: Charles River Breeding Laboratories, Inc., Portage, Michigan

The rat was selected as the test system because it is an acceptable model for acute toxicity studies.

Body Weight Range:
204-225 (males) and 152-171 (females) grams at day of dosing

Age at Start of Study:
Young adult, 8 weeks of age

Method of Identification:
Ear tag

Housing:

Individual hanging wire-mesh cages

Quarantine:

Maintained in accordance with the recommendations contained in the D.H.E.W. Publication No. 85-23 (N.I.H.) entitled "Guide for the Care and Use of Laboratory Animals" and conditioned for a period of 7 days prior to day of dosing.

Selection for Study:

Computer generated table of random numbers

Diet:

Certified Rodent Chow® #5002, Purina Mills, Inc., St. Louis, Missouri.

Environmental Conditions:

Animal room with controlled temperature, humidity and light (12 hours light and 12 hours dark). Diet and water freely available, except approximately 19 hours prior to dosing and three-four hours after dosing when diet, but not water, was withheld.

Dosing Solution:

The test articles were each administered as w/v suspensions in 0.5% aqueous methylcellulose.

Dosage Group (Level):

Each test article was administered to separate groups of five male and five female rats at a dosage level of 5,000 mg/kg.

Administration:

The test article was administered once by oral gavage using an appropriate sized, sterile, plastic syringe fitted with a 16 gauge, snub-tipped dosing needle. The dosage volume was 20 ml/kg.

OBSERVATIONS:

Mortality:

Observed 1, 2 1/2 and 4 hours after administration on the first day; twice daily thereafter for 13 days.

Pharmacotoxic Signs:

Observed 1, 2 1/2 and 4 hours after administration on the first day; once daily thereafter for 13 days.

Body Weights:

Immediately prior to administration, on day 8 and at study termination (day 15).

Necropsy:

Performed on all animals sacrificed at study termination (day 15) by qualified Acute Department personnel according to IRDC Standard Operating Procedure 44-1-4. No tissues were saved.

RESULTS:

Mortality:

All rats from each test article group survived to study termination.

LD_{50} Value:

Based on the results obtained, the LD_{50} value for each test article was estimated to be greater than 5,000 mg/kg when administered to fasted male and female rats.

Pharmacotoxic Signs (Appendix A):

Lot A-104

One male exhibited chromodacryorrhea within 1 hour post-dose on day 1 and persisted to day 3 and one female exhibited decreased activity at 4 hours post-dose on day 1. No other visible abnormalities were observed.

Lot DA01001

No visible abnormalities were observed in any animal during the study period.

Lot 1FL03E

One male exhibited high carriage within 2 1/2 hours post-dose, clearing by 4 hours post-dose. One other male and two females exhibited decreased activity within 4 hours post-dose and cleared by day 2. No other visible abnormalities were observed.

Body Weights (Appendix B):

There were no remarkable changes or differences observed in body weights during the study period.

Necropsy:

There were no visible abnormalities observed in any rat sacrificed at study termination from each test article group at the post-mortem examination. Necropsy examination results were not recorded for one animal in the Lot 1FL03E group. No tabular data are presented.

Technical Supervisory Staff,

Acute, Ocular and Dermal Toxicology:

Stephen W. Allen, B.S.
Group Supervisor

Gail Wagoner
Unit Supervisor

PREPARED BY: Gayle E Denaway 5/17/89

Gayle E. Denaway
Report Writer
Department of Report Writing

Date

REVIEWED BY: Dale E Johnson 5/18/89

Dale E. Johnson, Pharm.D., Ph.D., D.A.B.T.
Scientific Director, Experimental Toxicology Division

Date

STUDY DIRECTOR STATEMENT

The methods used in IRDC Study Number 538-002 followed the experimental criteria specified in the protocol.

To the best of my knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of this study. This study was conducted in conformance with the Good Laboratory Practice Regulations. This report accurately reflects the raw data obtained during the performance of this study.

All data including the final study report are stored in the International Research and Development Corporation Archives, Mattawan, Michigan.

James R. Myer 5/19/89

James R. Myer, B.S.
Manager, Acute, Ocular and Dermal Toxicology
Study Director

Date

APPENDIX A

Individual Clinical Findings

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset - Duration	Frequency
Algae Meal, Lot A-104:			
65723	No visible abnormalities	1a - 14	14
65724	No visible abnormalities	4 - 14	11
	Chromodacryorrhea	1a - 3	3
65730	No visible abnormalities	1a - 14	14
65733	No visible abnormalities	1a - 14	14
65748	No visible abnormalities	1a - 14	14

538-002

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = 1 hour after dosing, Day 1.

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Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset - Duration	Frequency
Algae Meal, Lot A-104:			
65754	No visible abnormalities	1a - 14	14
65756	No visible abnormalities	1a - 14	14
65763	No visible abnormalities	1a - 14	14
65767	No visible abnormalities	1a - 14	14
65772	No visible abnormalities	1a - 14	14
	Decreased activity	1c - 1c	1

538-002

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = 1 hour after dosing, Day 1.
c = 4 hours after dosing, Day 1.

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset - Duration	Frequency
Algae Meal, Lot DA01001:			
65722	No visible abnormalities	1a - 14	14
65731	No visible abnormalities	1a - 14	14
65742	No visible abnormalities	1a - 14	14
65743	No visible abnormalities	1a - 14	14
65747	No visible abnormalities	1a - 14	14

538-002

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = 1 hour after dosing, Day 1.

000221

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset - Duration	Frequency
Algae Meal, Lot DA01001:			
65768	No visible abnormalities	1a - 14	14
65769	No visible abnormalities	1a - 14	14
65770	No visible abnormalities	1a - 14	14
65771	No visible abnormalities	1a - 14	14
65776	No visible abnormalities	1a - 14	14

538-002

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = 1 hour after dosing, Day 1.

000222

Individual Clinical Findings
Male

Group, Rat Number		Day of Study Onset - Duration	Frequency
Algae Meal, Lot 1FL03E:			
65725	No visible abnormalities	1a - 14	14
65726	No visible abnormalities	1a - 14	14
65734	No visible abnormalities	1a - 14	14
	High carriage	1b - 1b	1
65740	No visible abnormalities	1a - 14	14
	Decreased activity	1c - 1c	1
65749	No visible abnormalities	1a - 14	14

538-002

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = 1 hour after dosing, Day 1.
b = 2 1/2 hours after dosing, Day 1.
c = 4 hours after dosing, Day 1.

000223

Individual Clinical Findings
Female

Group, Rat Number		Day of Study Onset - Duration	Frequency
Algae Meal, Lot IFL03E:			
65750	No visible abnormalities	1a - 14	14
	Decreased activity	1c - 1c	1
65757	No visible abnormalities	1a - 14	14
65764	No visible abnormalities	1a - 14	14
65766	No visible abnormalities	1a - 14	14
65773	No visible abnormalities	1a - 14	14
	Decreased activity	1c - 1c	1

538-002

Onset = Day first observed
Duration = Day last observed
Frequency = Number of days observed

a = 1 hour after dosing, Day 1.
c = 4 hours after dosing, Day 1.

000234

APPENDIX B

Individual Body Weights

Individual Body Weight, Grams

GROUP, ANIMAL NO.	SEX	OBSERVATION PERIODS (day) 0	8	15
Algae Meal, Lot 1FL03E				
65750	F	152	199	228
65757	F	155	194	221
65764	F	159	207	229
65766	F	164	211	230
65773	F	157	202	236

538-002

000227

Appendix 24

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KOYO MERCANTILE COMPANY, LTD.

CABLE ADDRESS
"KOYOMERCANTILE"
TOKYO

NAKAO BLDG.
12-16 NIHONBASHI-HONCHO 4-CHOME
CHUO-KU, TOKYO
103 JAPAN

TEL. TOKYO 03(639)8686
TLX. 0-252-2062
FAX. 03(667)8718

August 17, 1989

Dr. David W. Krempin
Microbio Resources, Inc.
6150 Lusk Blvd., Suite B-105
San Diego, Calif. 92121
U. S. A.

Dear Dr. Krempin:

Please find enclosed copies of English translation of the following:

1. Acute Toxicity Test of Alga Meal
   (Testing was made by Nippon Animal Feeding Corporation)

2. Mutagenicity Test of Alga Meal
   (Testing was made by Japan Food Analysis Center)

3. Japanese Patent Laid-Open No. 47359/1989

Thanking you, we are

Very truly yours,

KOYO MERCANTILE CO., LTD.

O. Kaede
President

OK/ik

Encl.

000228

ACUTE TOXICITY TEST

1. Client

   RIKEN VITAMIN K.K.

2. Test sample

   Alga meal (February 5, 1988)

3. Administration route

   Oral

4. Test animal

   ddv-N male and female mice.

   Age and body weight at initiation: ca. 5 weeks,
   male: 24g, and
   female: 20 - 22g.

5. Room temperature

   22 ± 2 °C

6. Test period

   From March 9 to March 15, 1988

7. Preparation of test solution

   30g of the test sample was suspended in distilled water for injection to give a total volume of 100ml (concentration of sample: 30% (w/v)).

8. Administration of test solution

1) Method

   Forced oral administration once by using a gastric probe.

2) Calculation of LD_{50}

   Probit method

000229

3) Administration concentration ratio of test sample

1 : 1.2

4) Number of animals per group

10 males and 10 females

9. Results

Sex	Test group No.	Dose (mg/kg)	Mortality change with time (hr) 5 15	Mortality change with time (day) 1 2 3 4 5 6 7	Mortality (%)	LD_{50} (mg/kg)
M	1	10,417	0/10 0/10	0/10 ..... 0/10	0	>18,000
	2	12,500	0/10 0/10	0/10 ..... 0/10	0	
	3	15,000	0/10 0/10	0/10 ..... 0/10	0	
	4	18,000	0/10 0/10	0/10 ..... 0/10	0	
F	1	10,417	0/10 0/10	0/10 ..... 0/10	0	>18,000
	2	12,500	0/10 0/10	0/10 ..... 0/10	0	
	3	15,000	0/10 0/10	0/10 ..... 0/10	0	
	4	18,000	0/10 0/10	0/10 ..... 0/10	0	

10. Symptom of intoxication

In each male or female group, a slight or moderate lowering in ultromotivity was observed approximately 10 minutes after the administration and dark red feces, presumally the excreted sample, were observed after approximately two hours. Each of these phenomena disappeared within approximately 48 hours and no

000230

abnormality was observed thereafter. No animal died.

11. Autopsy

No group showed any abnormality in major organs.

12. Discussion

In this test, neither any male nor femal animal died by the administration of the test sample in a dose of 18,000 mg/kg. The oral LD_{50} of the test sample for both of male and female mice was judged to be 18,000 mg/kg or above, since the dose of the test sample of 18,000 mg/kg corresponded to a dose of the test solution of 60 ml/kg and was thought to be the maximum level in the case of the forced oral administration and the concentration of the test solution was on the highest level available in the administration by using a gastirc probe.

The attached table shows the body weight of individual mouse employed in the test.

Address: 821 Yoshikura, Narita-shi, Chiba-ken

Testing Organization: Animal Feeding Research Center,
Nippon Animal Feeding Corporation

Testing Staff: Harunobu NORO, Takatoshi SHIMIZU, Senri YONEMOCHI,
Yuji KAZAHAYA and Hiroaki YAMAZAKI

OVER

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Appendix 25

RECEIVED AUG 21 1989

KOYO MERCANTILE COMPANY, LTD.

CABLE ADDRESS
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12-16 NIHONBASHI-HONCHO 4-CHOME
CHUO-KU, TOKYO
103 JAPAN

TEL. TOKYO 03(
TLX. 0-
FAX. 03(

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Dr. David W. Krempin
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Dear Dr. Krempin:

Please find enclosed copies of English translation of the following:

1. Acute Toxicity Test of Alga Meal
   (Testing was made by Nippon Animal Feeding Corporation)

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   (Testing was made by Japan Food Analysis Center)

3. Japanese Patent Laid-Open No. 47359/1989

Thanking you, we are

Very truly yours,

KOYO MERCANTILE CO., LTD.

O. Kaede
President

OK/ik

Encl.

000232

1. Client

RIKEN VITAMIN K.K.

2. Test sample

Alga meal (February 5, 1988)

3. Object of the Test

In order to examine the mutagenicity of this sample, a reversion test involving the evaluation of the activation of metabolism was conducted by using Escherichia coli WPS uvr A strain and five Salmonella typhimurium TA strains according to the Notification No. 261 of the Labor Standards Bureau, Ministry of Labor (May 18, 1985).

4. Test method

1) Test strains

Salmonella typhimurium TA100, TA 1535, TA98, TA1537 and TA1538 and E. coli WP2 uvr A strains were used.

Each strain was inoculated into 10 ml of nutrient broth No. 2 (OXOID) in an L-shaped test tube and cultured under shaking at 37°C for 10 hours prior to the initiation of the test.

2) Preparation of sample solution

The sample was sterilized since it was contaminated with bacteria and thus could not be tested as such. The test solution thus prepared was a suspension, which made it impossible to remove the bacteria by filtration.

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Thus the sample was subjected to direct high-pressure steam sterilization at 121°C for 10 minutes. The sterilized sample was accurately weighed and formulated into a 50 mg/ml suspension in dimethyl sulfoxide. The obtained suspension was appropriately diluted with dimethyl sulfoxide and subjected to the test.

3) Test procedure

The test was conducted through a preincubation technique under two conditions, namely, with and without added activating metabolism.

0.1 ml of the sample suspension, 0.5 ml of S9Mix*1 or 0.1 M sodium phosphate buffer solution (pH 7.4) and 0.1 ml of each bacterial suspension were successively introduced into a small sterilized test tube and shaken in a thermostat tank at 37°C for 20 minutes (preincubation). Next, 2 ml of top agar*2 was added thereto. The obtained mixture was uniformly spreaded on a minimum glucose agar plate medium*3 and solidified. Then it was cultured in a thermostat at 37°C for 48 hours and the colonies thus formed by reversion were counted.

It was confirmed that none of the bacterial suspension, sample suspension and S9Mix was contaminated with infectious microbes. Furthermore, a positive control test was conducted on the compounds shown in the appended tables.

000234

tryptophan solution was added to the E. coli WP2 uvr a strain, each in an amount of 1/10 by volume, and mixed.

*3: Composition of minimum glucose agar plate medium (per liter)

$MgSO_4 \cdot 7H_2O$	0.2 g
citric acid monohydrate	2 g
K_2HPO_4	10 g
$NaNH_4HPO_4 \cdot 4H_2O$	3.5 g
glucose	20 g
agar	15 g

30 ml portions of the medium were pipetted into sterilized plates of 100 mm in diameter and solidified.

5. Results of the test

As Tables 1 and 2 indicate, positive controls AF-2, 1-ethyl-2-nitro-3-nitrosoguanidine, 9-aminoacridine and 2-nitrofluorene showed each a remarkable increase in the number of revertant colonies, compared with the case of the solvent control. 2-Aminoanthracene induced remarkable reversion in the presence of S9Mix. In contrast to these results, the sample showed no significant increase in the number of revertant colonies in every case, compared with the solvent control.

These facts indicate that the mutagenicity of

000235

*1: Composition of S9Mix (per ml)

S9	0.1	ml
$MgCl_2$	8	μmol
KCl	33	μmol
G-G-P	5	μmol
NADPH	4	μmol
NADH	4	μmol
sodium phosphate buffer solution (pH 7.4)	100	μmol

S9 was purchased from Kikkoman Co., Ltd. and preserved at -80°C.

The data appended to S9 were as follows.

Test animal: male SD rats (age: 7 weeks, body weight: 222 - 260 g).

Inducer: phenobarbital (PB) and 5,6-benzoflavone (5,6-BF)

Dose: PB: 30 + 60 + 60 + 60 (mg/kg), 5,6-BF: 80 mg/kg.

Administration method: intraperitoneal injection.

Production date: December 18, 1987.

*2: Composition of top agar

Bacto agar (DIFCO)	0.6 %
NaCl	0.5 %

After the high-pressure steam sterilization, a 0.5 mM histidine/HCl H_2O-0.5 mM solution was added to each of the five Salmonella strains, while a 0.5 mM

000236

the sample under the presently employed conditions is negative.

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No. 41020585 Table 1: Results of the test Appendix 1

試験結果表 1

Sample: Alga meal
供試品：アルゲミール

Substance 物質	Concn. μg/plate μg/平板	S9Mix	No. of revertant colonies/plate 復帰変異コロニー数/平板					
			塩基対置換型 Base pair substitution type			フレームシフト型 Frame-shift type		
			TA100	TA1535	WP2uvrA	TA98	TA1537	TA1538
Solvent control 溶媒対照	0	−	104	6	14	13	10	6
			106	6	12	9	5	6
Sample 供試品	156	−	105	12	12	13	6	5
			123	7	12	16	3	4
	313		100	9	13	13	7	4
			119	9	9	10	8	6
	625	−	101	6	16	15	6	8
			102	14	11	15	6	11
	1250	−	90	9	16	13	6	3
			102	6	9	13	4	10
	2500	−	98	7	15	13	11	5
			114	7	12	20	3	5
	5000	−	102	13	17	16	1	8
			104	9	9	14	2	5

Positive control 陽性対照 Name 名称

S9Mix		AF-2	ENNG	AF-2	AF-2	9-AA	2-NF
(−)	濃度 μg/平板	0.01	5	0.01	0.05	80	2
	コロニー数/平板	316	1274	136	266	412	299
	Concn. (μg/plate)	364	1344	118	277	520	218
	No. of colonies/plate						

AF-2 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide
ENNG 1-ethyl-2-nitro-3-nitrosoguanidine
9-AA 9-aminoacridine
2-NF 2-nitrofluorene

000238

Appendix 26

Gross Pathologic Examination of Salmonids from Dietary Study with NatuRose Natural Astaxanthin (Haematococcus algae meal)

Prepared for
Cyanotech Corporation

by
Dr. Jan Spitsbergen, DVM, Ph.D.

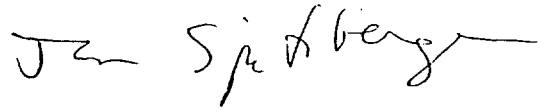
5/8/97

Results of Gross Examination of Fish Tissues

Fish tissues were received well packed on cold packs via Federal Express from Dr. Ron Hardy of Hagerman Fish Culture Experiment Station, Idaho, on 4/17/97 and were cold upon arrival. Viscera including stomach, liver and intestines were present from fish for Diet 1 (control diet). For Diet 2, viscera and heads of 6 fish were present. For diets 3, 4 and 5, heads with spine, kidney, some skeletal muscle, caudal fin and viscera were present for 10 fish per diet. The shape, consistency and color of all tissues were evaluated grossly to look for parasites, toxic effects or neoplasia. All tissues examined were normal in appearance, with no indication of disease, toxicity or neoplasia. All fish examined were in excellent nutritional status with abundant body fat. It must be emphasized that gross examination can not rule out the possibility of subtle, microscopic changes in tissue. Histologic examination would be required to rule out subtle changes.

Conclusions:

Initial gross findings indicate no adverse effects of the NatuRose natural astaxanthin (Haematococcus algae meal) preparation on fish health. Histologic study of fish tissues will be required to rule out possible subtle effects on fish tissues.

Jan M. Spitsbergen 

Appendix: Biographical Sketch of Dr. Spitsbergen

Address: Department of Food Science and Technology
Wiegand Hall
Oregon State University, Corvallis OR 97331

Phone: 541-737-5055

EDUCATION:

Michigan State University, B.S. in Fisheries and Wildlife, March 1976. Major field of study fisheries and limnology, minor biochemistry.

Michigan State University College of Veterinary Medicine, D.V.M., June, 1980.

Cornell University, Ph.D. in immunology and pathology, January, 1986. Minor field of study toxicology.

RESEARCH AND PROFESSIONAL EXPERIENCE:

1980 Aquavet, a course in aquatic veterinary medicine, Marine Biological Laboratory, Woods Hole, MA.
1980 Research Associate, Marine Biological Laboratory, Woods Hole, MA.
1980-1982 Intern, then resident, Department of Veterinary Pathology, Cornell University, Ithaca, NY.
1982-1986 Research Assistant (Ph.D. Candidate) in Departments of Veterinary Pathology and Avian and Aquatic Animal Medicine, Cornell University, Ithaca, NY.
1986-1988 Research Associate, School of Pharmacy, University of Wisconsin, Madison
1988-1995 Assistant Professor, Department of Avian and Aquatic Animal Medicine, Cornell University, Ithaca, NY.
1990 Recombinant DNA Techniques Workshop, Life Technologies, Inc., Germantown, MD.
1991 Morphometry in Pathology and Toxicology Short Course, Princeton, NJ.
1993 Microinjection Short Course at Marine Biological Laboratory, Woods Hole, MA.
1993 Participated in NIH Study Section regarding use of small aquarium fish in carcinogenesis studies.
1995-present Research Associate, Department of Food Science and Technology, Oregon State University, Corvallis, OR

HONORS, AWARDS, CERTIFICATIONS AND PROFESSIONAL ACTIVITY

Phi Zeta, June, 1979; Catherine Patton Award in Veterinary Physiology, June, 1978; Phi Kappa Phi Honor Society, March, 1976; Diplomate, American College of Veterinary Pathologists, 1987; Most Significant Paper in Journal of Aquatic Animal Health in 1995 (7:269-283); Member of American Veterinary Medical Association, Fish Health Section of American Fisheries Society, Society of Toxicologic Pathologists

SELECTED REFERENCES

SPITSBERGEN, J.M., SCHAT, K.A., KLEEMAN, J.M. and PETERSON, R.E.. (1986). Interactions of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) with immune responses of rainbow trout. *Vet. Immunol. and Immunopathol.* 12,263-280.

SPITSBERGEN, J.M., KLEEMAN, J.M. and. PETERSON, R.E. (1988). Morphologic lesions and acute toxicity in rainbow trout (Salmo gairdneri) treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *J. of Toxicol. Environ. Health* 23,333-358.

SPITSBERGEN, J.M., KLEEMAN, J.M., SCHAT, K.A. and PETERSON, R.E.. (1988). Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or Aroclor 1254 on the resistance of rainbow trout, Salmo gairdneri Richardson, to infectious hematopoietic necrosis virus. *J. Fish Dis.* 11, 73-83.

SPITSBERGEN, J.M., J.M. KLEEMAN and PETERSON, R.E. (1988). 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity in yellow perch Perca flavescens. *J. of Toxicol. Environ. Health* 23:359-383.

SPITSBERGEN, J.M., Walker, M.K.,Peterson, RE. and Olson, J.R.. (1991). Pathologic alterations in early life stages of lake trout exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Aquat. Toxicol.* 19,41-72.

WALKER, M.K., SPITSBERGEN, J.M.,OLSON, J.R. and PETERSON, R.E.(1991). 2,3,7,8-tetrachlorodibenzo-p- dioxin (TCDD) toxicity during early life stage development of lake trout (Salvelinus namaycush). *Can. J. Fish.Aquat.Sci.* **48**, 875-883.

POULET, F.M., CASEY, J.W. and SPITSBERGEN, J.M. (1993). Studies on transmissibility and etiology of orocutaneous tumors of brown bullheads (Ictalurus nebulosus). *Dis. Aquat. Org.* **16**, 97-104.

POULET, F.M., WOLFE, M.J. and SPITSBERGEN, J.M. (1994). Naturally occurring orocutaneous papillomas and carcinomas of brown bullheads (Ictalurus nebulosus) in New York State. *Vet. Pathol.* **31**, 8-18.

FISHER, J.P., SPITSBERGEN, J.M. and JAHAN-PARWAR, B.. (1994). Effects of embryonic PCB exposure on hatching success, survival, growth and developmental behavior in landlocked Atlantic salmon, Salmo salar. In *Environmental toxicology and risk assessment*, 2nd Vol. ASTM STP **1173**. (J.W. Gorsuch, F.J. Dwyer, C.G. Ingersoll and T.W. La Point, eds), pp. 298-314. American Society for Testing and Materials, Philadelphia, PA.

SPITSBERGEN, J.M. AND WOLFE, M.W. (1995). The riddle of hepatic neoplasia in brown bullheads from relatively unpolluted waters in New York State. *Toxicol. Pathol.* **23**, 716-725.

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